

MODEL PROJECT INFORMATION SHEET
EARLY DIAGNOSIS OF CONGENITAL DISEASES IN CHILDREN
URUGUAY - URU/6/022

SUMMARY

In Uruguay congenital diseases are at present the second principal cause of death in children under one year of age. Failure to diagnose and treat some congenital diseases early can lead to irreversible damage that impairs the child for its entire life. Two such diseases are neonatal hypothyroidism and cystic fibrosis. If left untreated, the result can be severe: irreversible mental retardation in the case of neonatal hypothyroidism, and repeated severe respiratory infections and disorders of the digestive function in the case of cystic fibrosis. Radioimmunoassay (RIA) of blood samples is the most effective means of diagnosing hypothyroidism, and cystic fibrosis can be detected through application of the polymerase chain reaction technique and phosphorus-32 labelled DNA probes. The Government has made screening of newborn infants for hypothyroidism mandatory, and a national screening programme will be carried out under this model project.

The RIA laboratory of the Nuclear Medicine Centre in Montevideo will be the national reference laboratory and the Centre will co-ordinate activities involving other national institutions. Two additional diagnostic centres will be established in the northern and eastern parts of the country. For cystic fibrosis, improved diagnostic techniques will be introduced at the Cytogenetics Laboratory of the Institute of Biological Research to study the incidence of the disease in Uruguay and to establish diagnostic procedures for a future screening programme.

Project duration: 2 years. Total budget: US \$154,280.

INTRODUCTION

Congenital diseases are defined as the presence at birth of morphological and/or functional defects or disorders, which cause dysfunction of varying severity related to the

organs and systems concerned. Most of the major congenital diseases such as chromosomal and genetic abnormalities and foetal diseases by malformation or agenesis are accompanied by mental retardation. These include metabolic and hormonal production diseases whose adverse consequences on the neuropsychological development of the child can be avoided by early neonatal diagnosis and specific treatment. Congenital diseases for which treatment is available and which result in a clear social and economic benefit include neonatal hypothyroidism and cystic fibrosis.

Neonatal hypothyroidism is deficiency or dysfunction of the thyroid hormones (T_3, T_4) during the early life of newborns. The disease is most prevalent in areas of endemic iodine deficiency. In the most serious cases, the condition may lead to irreversible neurological impairment or deaf mutism, but varying degrees of mental and intellectual impairment ensue even when the iodine lack is less severe. The problem is therefore of critical public health concern and of great socioeconomic importance to developing countries, where iodine deficiency disorders are very common. Neonatal hypothyroidism could occur in as many as 1 in 1000 live births. The cost of a screening programme would be insignificant compared to the cost to the family or the community of maintaining even a few severely mentally retarded, socially unproductive persons, especially as some of them may need institutional care. Neonatal hypothyroidism is treatable provided it is detected early enough, i.e. within the first few days of life.

Cystic fibrosis (CF) is the most common lethal autosomal recessive disorder affecting Caucasians, with a disease frequency as high as 1 in 2500 and an estimated frequency of 1 in 25. It is characterized by functional abnormalities in the airway epithelium, the exocrine pancreas, the gastrointestinal tract, and the secretory duct of sweat glands, leading to pancreatic and pulmonary insufficiency. Defective transport of chloride ions across epithelia is responsible for many of the symptoms of CF. The inadequate secretion of chloride and excessive resorption of sodium cause insufficient hydration of mucus in the airways and pancreatic ducts. This leads to frequent bacterial infections in the airways and obstruction of the pancreatic ducts, causing permanent pancreatic insufficiency. There is considerable variability in the age of onset, the severity of the disease, and the rate of progression of the involved organs, with symptoms progressing many years before some individuals are brought to clinical attention. There have been remarkable advances in CF research and a rapidly evolving understanding of the pathogenesis of the disease in the past few years, leading to new strategies for immediate therapeutic intervention. Thus, screening for CF in the newborn period to avoid delays in essential treatment is warranted.

In Uruguay the two main causes of death in children under the age of one year are at present, firstly, birth traumatism, anoxia and other disorders of the foetus of newborn babies (20.9% of infantile deaths) and, secondly, congenital abnormalities (19.4% of infantile deaths)¹. Acute respiratory and intestinal infections as a cause of death have moved down to fourth and seventh place (7.8% and 3.2% of infantile deaths) respectively during the last ten years as a consequence of the implementation of a primary and secondary prevention programme for these disorders. Malnutrition occupies tenth place, accounting for 2% of the total number of deaths of children under the age of one. In order to reduce the relatively high

¹Statistics Ministry of Public Health

incidence of congenital diseases as cause of disease and mortality at and after birth, Uruguay has adopted a specific approach which includes:

- (a) Mandatory screening for neonatal hypothyroidism.
- (b) Implementation of a National Screening Programme to extend the screening for neonatal hypothyroidism to the different regions of the country. Participating in this programme will be the Nuclear Medicine Centre from the Clinical Hospital of the University of the Republic, the Social Security Bank (BPS) and the Nuclear Research Centre (CIN). According to statistics of the Ministry of Public Health, the number of births per year in Uruguay is about 55,000 of which about 21,000 occur in Montevideo and the rest (around 60%) are distributed in different regions of the country. It is necessary to strengthen existing diagnostic capabilities to ensure extension of the screening to the whole neonatal population. Under an agreement between the Ministry of Public Health, the Nuclear Medicine Centre and the Faculty of Medicine, it is planned to establish two regional diagnostic centres, one in the north, in the Department of Salto, and the other in the east, in the Department of Maldonado, near Montevideo. Co-ordination with the National Vaccination Programme is envisaged for transport of samples, supervision of treatment of positive cases and general sanitary surveillance of the programme.
- (c) Implementation of a Multidisciplinary Study for the Diagnosis and Prevention of Congenital Anomalies in Paediatrics. This is a long term (six years) project initiated in 1994 in which the Institute of Paediatrics, the Cytogenetics Division of the Institute of Biological Research (IIBCE) of the Ministry of Education and Culture, the Faculty of Medicine and the Nuclear Medicine Centre are participating. Its main objectives are to determine the incidence of congenital diseases in Uruguay, to improve existing diagnostic techniques and to introduce new procedures, and to train the multidisciplinary teams that will conduct clinical and basic research on congenital diseases and will participate in prevention programmes and in the diagnosis, treatment and early rehabilitation of children.

CURRENT NATIONAL PROGRAMME

There are two ongoing programmes for neonatal hypothyroidism, one at the Nuclear Medicine Centre of the Clinical Hospital of the University of the Republic and the other at the Social Security Bank (BPS), both activities being linked as part of a National Screening Programme. The programme of the University started in 1990 and that of the BPS in 1992. By early 1994, 21,500 newborn babies from Montevideo and other parts of the country had been tested at both centres. Seven positive cases were detected and given timely treatment, thus avoiding the adverse consequences of the disease for the children's lifetime as well as for the families and the community.

Previous Agency assistance through the ARCAL projects RLA/6/011 (Radioimmunoassay of Thyroid-Related Hormones) and RLA/6/016 (Production of Radioimmunoassay Reagents) made an important contribution to the introduction of radioimmunoassay (RIA) techniques and local production of reagents for RIA kits and training.

The counterpart institutions of this model project are the Nuclear Medicine Centre and the Cytogenetics Laboratory of the IIBCE, in co-ordination with the Social Security Bank, the Nuclear Research Centre, the National Vaccination Programme and the Department of Epidemiology of the Ministry of Public Health.

In the case of cystic fibrosis, the studies carried out so far by the IIBCE's Cytogenetics Laboratory for the eight most frequent and serious mutations show that in a significant number of cases it was possible to detect only one mutation in the individuals affected. This could indicate the probable existence of other mutations (apart from the eight analysed) in the genesis of cystic fibrosis in Uruguay. Similarly, mutation ΔF_{508} , which is present in almost 68% of European individuals affected, was found in a much smaller proportion in a relatively small sample of individuals analysed in Uruguay. This would suggest that the distribution of mutations of the gene for cystic fibrosis in the European population cannot be extrapolated to the population of Uruguay which, although the majority is of European origin, is mixed with indigenous and African populations. Currently, the IIBCE is studying improved diagnostic techniques in order to determine the incidence of the disease in Uruguay and to establish diagnostic procedures for future screening programmes.

NUCLEAR TECHNOLOGY

For **neonatal hypothyroidism**, the best approach for screening is by measuring the thyroid related hormones, T_4 and TSH, in the baby's blood by RIA methods. For screening purposes, the blood is obtained on the fourth day of life from a heel-prick on to a filter paper strip. Babies suspected to be hypothyroid are immediately recalled for further testing, and treatment is given with minimum delay. The RIA tests are simple and inexpensive enough to be applied on a wide national scale, thus underscoring the cost- effectiveness and economic impact of a screening programme, especially as it could be combined with screening for other inherited disorders such as phenyl ketonuria and cystic fibrosis.

Earlier newborn screening strategies for **cystic fibrosis (CF)** were based on meconium albumin levels, which are elevated in CF infants. The test, however, has low specificity and gave a high false negative rate. In 1979, an RIA-based test was developed for measuring immunoreactive trypsinogen level in the new born. The test has better sensitivity and specificity, and can detect up to 90% of patients with CF.

Since the identification of the CF transmembrane conductance regulator (CFTR) gene in 1989, DNA-based tests have been developed to identify patients with CF. The principal mutation in the CFTR gene is a three base pair deletion referred to as ΔF_{508} . It is reported to account for 50%, 70% and 80% of CF heterozygotes in Italy, Belgium and the United Kingdom, respectively. Although some 170 additional mutations have been identified, only three of these (G542X, N1303K, 1717-IG) are important, accounting for an additional 10-12% of CF carriers. Primers have been developed that allow the simultaneous amplification of several of these mutations, including the four major ones, by polymerase chain reaction (PCR). The amplified products are then detected by agarose gel electrophoresis and by the use of ^{32}P -labelled probes with sequences complementary to the sequence of the mutations. Non-radioactive probes may also be used but their sensitivity is much lower than radioactive probes. There are no other appropriate techniques.

OBJECTIVES

1. To create a capability by which every newborn in the country would be screened for neonatal hypothyroidism and cases detected as positive would be treated promptly enough to prevent the development of sequelae such as mental retardation.
2. To establish facilities for the use of radionuclide-based DNA techniques for prenatal and postnatal screening of cystic fibrosis.

PROJECT IMPLEMENTATION AND MONITORING

Neonatal hypothyroidism: During the first year of the project, it is planned to establish a diagnostic centre in the Department of Salto. The basic infrastructure and qualified medical staff will be made available by the Ministry of Public Health and the Faculty of Medicine. Equipment, reagents and supplies will be provided, together with expert assistance to introduce relevant technology and to organize a national training course on RIA techniques for the staff involved in the National Screening Programme. In the second year, another diagnostic centre will be established in the Department of Maldonado. With these two additional facilities it is expected to provide diagnosis for all newborns. The RIA laboratory of the Nuclear Medicine Centre in Montevideo will be the national reference laboratory. An external quality control programme will be established in order to ensure reliability of the results. However, each laboratory will have its own internal quality control procedures as part of standard RIA practice. The samples (filter paper blood spot) will be collected at the moment of birth. In Uruguay, the majority of births (99.6%)¹ take place in maternity hospitals or primary health centres, so that this procedure can be easily implemented. The National Vaccination Programme will collaborate in the transport of samples and supervision of treatment of positive cases. This is a very strong organization with about 200 well trained staff, able to reach the entire population of newborns, including those born at home, with the antituberculosis vaccination campaign.

Cystic fibrosis: During the first year of the project, equipment, reagents and supplies will be provided together with expert advice to introduce radionuclide-based DNA techniques at the Cytogenetics Laboratory of IIBCE. Local staff will be trained to initiate the screening programme for neonates in Montevideo. During the second year, it is planned to extend the screening of neonates to other regions and to adult carriers in Montevideo.

The Nuclear Medicine Centre will be responsible for co-ordinating project activities with the local institutions involved. Implementation will be monitored by an annual progress report submitted to the Agency and discussed at annual meetings attended by Agency representatives and all participating and interested parties. Upon completion of the project, a seminar will be held for final evaluation, including discussion of the results and quantitative assessment of the impact, including cost-benefit analysis and recommendations for the future. A model project final report will be prepared by the Agency on the basis of the seminar papers.

NATIONAL COMMITMENT

The issuance of Decree No. 183/94 in September 1994, which makes mandatory the screening for neonatal hypothyroidism, is a clear commitment of the Government to reduce the rate of morbidity and mortality caused by congenital diseases in children. The direct recipient institute, the Nuclear Medicine Centre, has competent staff and a well established RIA laboratory. Infrastructure and qualified personnel are available for the establishment of regional diagnostic centres for neonatal hypothyroidism. The Cytogenetic Laboratory of the IIBCE has facilities for cell culture, microscopy and molecular biology as well as staff, and will provide operational support.

THE AGENCY'S INPUT

The Agency will provide expert services to assist the counterpart in organizing the national screening programme for neonatal hypothyroidism, for the introduction of RIA techniques at the regional diagnostic centres and for the diagnostic techniques for cystic fibrosis at the IIBCE. Equipment, reagents and supplies as well as staff training through a national training course, fellowships and scientific visits will also be provided.

IMPACT

The project will contribute significantly to the improvement of public health, particularly for children, and emphasizes equity. The National Screening Programme for neonatal hypothyroidism will allow the early detection of the disease and timely treatment for positive cases. The economic cost of the screening would be insignificant compared to the socioeconomic cost of maintaining mentally retarded persons. In Uruguay, the cost per sample is approximately US \$3. Occurrence of the disease in the country is estimated to be 1 in 3500, so that the cost of detecting one positive case would be US \$10,500. Institutional care for a child costs about \$3000 per year, and average survival period of individuals affected is about 30 years. Consequently, the cost of care for one person could reach US \$105,000. Only in economic terms, cost-benefit would be 1 to 10 in the conditions of Uruguay. The introduction of radionuclide-based DNA technique would improve the diagnosis for cystic fibrosis and would allow the future implementation of screening programmes in the country. Moreover, this technique can be utilized for the detection of other congenital diseases such as phenyl ketonuria and Down's syndrome.

FINANCES

The budget allocation for the project is US \$154,280, distributed as follows:

Year	Experts		Equipment	Fellowships		Scientific Visits		Grp trg.	Sub- contr.	Misc. Comp.	Total
	MD	US \$	US \$	MD	US \$	MD	US \$	US \$	US \$	US \$	US \$
1995	1/21	19,380	84,000	3/0	9,900	1/0	12,600	0	0	0	125,880
1996	0/21	8,400	20,000	0/0	0	0/0	0	0	0	0	28,400
Total	2/12	27,780	104,000	3/0	9,900	1/0	12,600	0	0	0	154,280

Source of funding: TACF